Original Research Article

In Vitro* Anthelmintic Activity of Mahaneem (*Melia azedarach*) and Chirata (*Swertia chirata*) extracts against eggs and adult stage of *Haemonchuscontortus

ABSTRACT

Aim: This study aimed to evaluate *in vitro* anthelmintic activity of Mahaneem leaves (*Melia azedarach*) and Chirata whole plant (*Swertia chirata*).

Study Design: *In vitro* study were conducted aganist*Haemonchuscontortus* eggs and adult stage by egg hatch assay (EHA) and adult mortality or motility inhibition test (AMIT).

Place and duration of study: This study were conducted in the departement of Veterinary Parasitology ,WBUAFS,Kolkata-37 between November,2023 to August, 2024

Methodology: Mahaneem leave and Chirata whole plant were collected,wash and dry avoiding direct sunlight. Then aqeous extract by decoction method were prepared, dried anddesolved in 2% Dimethyl Sulfoxide to make desirable concentration of extract solutions. Adult *H.contortus* were collected from freshly slaughter abomasum sheep for separation of eggs and active motile adult used for AMIT.

Results: The result revealed that chirata extract had better efficacy against both eggs and adult stage of *Haemonchuscontortus* compare to mahanimbo leave extract. In EHA, chirata extract efficacy @ 50 mg , 25 mg and 10 mg/ml concentration were 85.599 ± 2.389, 60.180 ± 0.878, 39.954 ± 0.690 percent respectively. Whereas, Mahaneem at same concentration shown 77.442±0.963, 54.178±0.848, 35.898 ±1.328 percent efficacy respectively in EHA against *H.contortus* eggs. But @ 50 mg/ml and 25 mg/ml concentration chirata extract were statistically significantly ($P<0.05$) more efficacy than Mahaneem. In AMIT, also Chirata shows significantly more efficacy @ 50 and 25 mg/ml,but @10 mg Mahaneem shows better efficacy (38.88 ± 2.22) than Chirata (31.11 ± 2.22) extract. At 50 mg/ml concentration after 10 hours of experiment Chirata shows higest efficacy (82.22 ± 2.22) compare to Mahaneem (57.77 ± 2.22).

Conclusion: It may be concluded that both Chirata and Mahaneem aqueous extract have the anthelmintic activity at different concentration with variable results. They may be utilised after proper doseing against *Haemonchuscontortus*in small ruminats .

Keywords: Chirata, Mahaneem, Anthelmintic,*Haemonchuscontortus*, EHA, AMIT

1. INTRODUCTION

Haemonchosis can result in large economic losses by causing appetite depression, damages to gastric function, and alterations in total protein content, energy, and mineral metabolism of livestock (Zarlenga et al., 2016) and the control has relied on the use of synthetic anthelmintics leads to growing anthelmintic resistance against commonly avilable drugs (McRay et al.,2015). In addition, there has been an increasing concern over chemical residues in edible animal products associated with the use of anthelmintic drugs in livestock (Waller, 1997). In this scenario, It is an importants to search alternative of anthelmintic to control the parasitic problem in the animal husbandry sector. Studies to find alternative strategies for the control of nematodes have focused on various options, one of the strategy is to explore the anthelmintic properties of plants containing bioactive compounds such as secondary metabolites. In context, to these approaches is the exploration and screening of various plants for novel anthelmintic compounds (Hernández-Villegas et al., 2011). Anthelmintics derived from indigenous plants have pragmatic features for small hold farmers who cannot afford the commercial product, and for large commercial farmers who are shifting to organic farming (Gradé et al.,2008; Taylor et al., 2001). Furthermore, herbal anthelmintics as a natural product are implicated to least likely bioaccumulate in the tissues of animal and the environment (Taylor et al.,2011). They are likewise considered eco-friendly and biodegradable (Hammond et al.,1997; McCorkle et al.,1995). Above all, multiple bioactive compounds present in herbal anthelmintics may translate to multiple mechanisms in killing the parasites, which then limit the likelihood of developing anthelmintic resistance (Chagas, 2015). Since ancient times, people have been exploring the nature particularly medicinal

plants in search of new drugs. Medicinal plants are used by 80% of the world population for their essential health needs and this efficacy depends upon the current knowledge about taxonomic features of plant species, plant parts and biological property of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites (Vinoth et al.,2011). Screening for effective anthelmintic compounds remains a major obstacle in the drug development process and screening in the natural hosts is typically very expensive, requiring appropriate facilities and can raise concerns about animal welfare (Kumarasingha et al.,2014). So, In viitro examination may be an good option for priliminary study of different herbal anthelmintic activity .

2. MATERIALS AND METHODS

2.1. **Plant Materials Collection:** .Two ethnomedicinal plants Mahanimba or the beed tree leaves (*Melia azedarach*) and Chirata or bitter stick whole plant *(Swertia chirata*) were collected from in and around the WBUAFS,Kolkata and Mohanpur campus for in vitro anthelmintic activity screening on different parasitic stags of *Haemonchuscontortus*.Then all materials was washed 3 times with clean water and dry at room temperature for 7 to 15 days depend on materials avoiding direct sunlight. Then all the material was powdered seperately by motor grinder and measure desirable amount for preparation of extract. Plants were identified as per available literature on identification.

2.2. Aqueous Extract Preparation (Decoction):

About 100 gm finely powder was kept in 500 ml distulled water for overnight and next day boil for 30 minutes. Resulting after boiling decoction solution was cooled and filtered with Whatman's filter paper no-1 to collect the acquoussolution.Then extraction solution was evaporated and concentrated by lyophiliser (Simeco,India) and further dry by keeping at 40^0C in hot air oven. Dry weight measure and dissolved in 2 % dimethyl sulfoxide (DMSO) as per required concentration and store at 4^0c for future uses.

2.3. **Recovery of Adult H. contortus Worms and Eggs**

For egg hatch assay (EHA) eggs were recovered from adult female*H.contortus* and adult female worms were colleted from the abomasum of slaughtered sheep collected from the local abattoir (New Market,Kolkata). Adult female H. contortus were recovered from abomasum and washed three times in normal saline. Then collected active motile alive adult worms were used for adult motile inhibition test (AMIT) and others adult female*H.contortus* were triturated in a clean pestle and mortar to obtain the eggs. For egg hatch assay, recovery of eggs from eggs containg triturated materials and test procedure was done as per method described Cole et al.,(1992) with mild modification. The eggs were recovered with saturated saline solution by flotation and washed repeatedly in distilled water to get final aqueous egg suspension from triturated materials. Eggs were separated, quantified and used within 90 minutes for the test.

2.4. Egg Hatch Assay (EHA)

The guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) was followed for conducting EHA (Coles et al.,1992). Approximately 50 numbers of *H. contortus* egg suspension in 100 µl of distilled water was incubated with different concentrations (1, 2.5, 5,10, 25, 50 mg/mL) of each plant extract in 2% DMSO in a 96 flat-bottomed micro titre plate to obtain a final tested concentration of 1 to 50 mg/ml. Albendazole served as a positive control and was dissolved in 2% DMSO in de-ionized water to obtain a concentrations of 50 µg/ml and 20 µg/ml), while 2% DMSO and PBS served as the negative control. The setup was incubated in triplicate for each ex-tract at 27^0C for 48 hours. At the end of 30 hours, a drop of Lugol's iodine solution was added to each well and the number of larvae vs unhatched eggs (including larvated ones) was counted with an inverted microscope to calculate egg hatch inhibition. All experiments were under-taken in triplicate on three separate occasions

The percentage inhibition of egg hatching was calculated using the formula by Cala et al., (2012):

$$\text{Inhibition of egg hatching (\%)} = \frac{(\text{Eggs}+\text{L})-\text{L}}{(\text{Eggs}+\text{L})} \times 100$$

L = Number of larvae in a particular well.

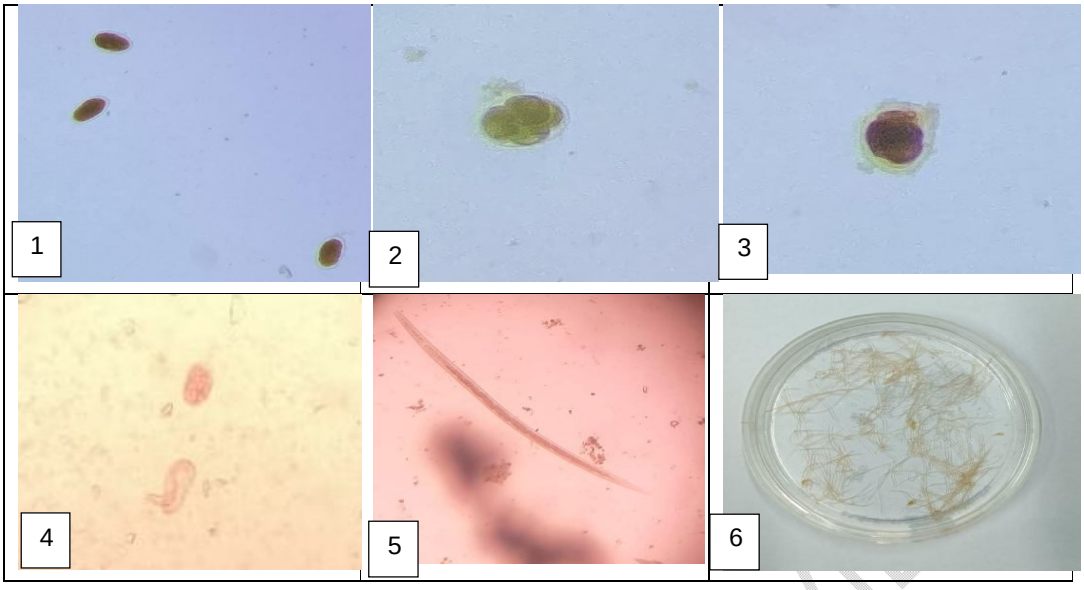

Fig.(1-6): 1. unhatched eggs of *H.contortus*treated with Chirata extract (50mg/ml); 2. Morola stage of *H.contortus* egg treated with Mahaneem extract (25 mg/ml); 3. unhatched larve stage 1 (L1) of *H.contortus* with uneven shaped egg shell; 4. Newly hetched L1 and unheatched egg of*H.contortus* in Chirata extract (10 mg/ml);5. Larvae stage 2 (L2) of *H.contortus* in Mahaneem extract (5mg/ml) after 48 hours 6. Adult active motile *H.contortus* collected for experiment

2.5. **Adult Worm Motility Inhibition (%WMI) Assay:** The AWMI test was performed in 50 mm diameter glass Petri dish according to Sharma et al.,(1971). Adult H. contortus worms were recovered from the sheep abomasums at laboratory collected and brought from local slaughter house (New Market, Kolkata). Then washed the worms thrice with PBS (pH ,7.2) and 10 actively motile worms were exposed thrice in three replicates to each plant extracts (50, 25, 10 and 5 mg/mL) in separate Petri dishes at temperature (28 ±1°C). Positive (albendazole @ 0.20mg /mL) and negative controls (worms with PBS) were included in the assay. The inhibition of motility and/or mortality of the worms exposed to the above concentrations was used as an indicator for anthelmintic activity. The motility of worms was

Concentration	Extract

observed by examination under a dissecting microscope at magnification x20 at intervals of 4 h till the worms in negative control lost their motility, for 10 h of assay. Finally, the extracts and albendazole were washed away and the worms were re-suspended in lukewarm fresh PBS for 30 min to observe and test the revival of motility. All the motile (alive) and immotile (dead) worms in three replicates of each concentration and control were counted. Death of the worms was ascertained by the absence of motility for observation period of 5–6 s. Percent

(mg/ml)	Mahaneem leaves	Chirata whole plant	p value
50 mg	77.442±0.963	85.599 ± 2.389	p<0.05
25mg	54.178± 0.848	60.180 ± 0.878	p<0.05
10 mg	35.898 ± 1.328	39.954 ± 0.690	p>0.05
5 mg	24.134 ± 0.773	18.295 ± 1.511	p<0.05
2.5 mg	8.437 ± 0.543	7.610 ± 0.561	p>0.05
1 mg	5.015 ± 0.198	4.967 ± 0.151	p>0.05
2 % DMSO	2.44 ± 0.08	3.12 ± 1.07	p>0.05
Albendazole-			
50 µg/ml	100 ± 0.00	100 ± 0.00	p>0.05
20 µg/ml	93.09 ± 1.18	93.40 ± 0.97	

Extract	Concentration	Time post exposure

2.6. worm motility inhibition (%WMI) was calculated as per Rabel et al.,(1994).
Dose-dependent % AWMI

$$= \frac{\text{No.of motile worms in negative control} - \text{No.of motile worms in treatment}}{\text{No.of motile worms in negative control}} \text{ X100}$$

2.6. Statistical Analyses

Comparison of mean percentages of egg hatch inhibition and larval paralysis at different concentration with the control was performed by one-way ANOVA by using SPSS IBM Statistics® v. 20.

3. RESULTS AND DISCUSSION

In this present study two ethnomedicinal plants parts Mahanimba or the beed tree leaves (Melia azedarach) and Chirata or bitter stick leaves and stems (Swertia chirayita) aquous extract were prepare for in vitro anthelmintic activity study at different concentration. In vitro study were conducted aganistHaemonchuscontortus eggs by egg hatch assay (EHA) and aganist adult stage of Haemonchuscontortus by adult mortality or motility inhibition test (AMIT).

The result revealed that chirata leave extract have better efficacy against both eggs and adult compare to mahanibbo leave extract. In EHA, chirata extract efficacy @ 50 mg ,25 mg and 10 mg/ml were 85.599 ± 2.389, 60.180 ± 0.878, 39.954 ± 0.690 respectively more compare to mahaneem at same concentration with 77.442 ± 0.963, 54.178 ± 0.848,35.898 ± 1.328 respectively (Table 1). But @ 50 mg and 25 mg concentration chirata extract statistically significant ((P=0.05)) more efficacy were seen. But @5 mg to 1 mg/ml concentration mahaneem shown more efficacy than chirata and statiticallysignicant only seen @ 5 mg/ml concentration.

Table 1. Mean efficacy (percentage±S.E) of aquoues extract of Mahanimba leaves (*Melia azedarach*) and Chirata (*Stewariachiratea*) on *H. contortus* egg hatching

In AMIT also chirata shows significantly (p<0.05) more efficacy @ 50 and 25 mg/ml than mahaneem (Table 2) , but @10 mg Mahaneem shows better efficacy (38.88 ± 2.22) than chirata (31.11 ± 2.22). Chirata extract @ 50 mg/ml after 10 hours experiment for AMIT shows highest efficacy with 82.22 ± 2.22 percent compare to Mahaneem with 57.77 ± 2.22 percent.

Table 2. Mean efficacy (percentage±S.E.) of aquoues extract of Mahanimba leaves (*Melia azedarach*) and Chirata (*Stewariachiratea*) on adult *H. contortus* motility.

		1 hour	3 hours	6 hours	10 hours
Chirata	50	24.44±2.22	31.11±2.22	51.11 ± 2.22	82.22±2.22
Whole	25	15.55±2.22	22.2 ±2.22	35.55 ± 2.22	51.11±2.22
plant	10	8.88 ± 2.22	11.11±2.22	22.22 ± 2.22	31.11±2.22
	5	6.66 ±0.00	8.88 ±2.22	15.55 ± 2.22	22.22±2.22
Mahan-	50	15.55±2.22	22.22±2.22	37.77 ± 2.22	57.77±2.22
eem	25	11.11±2.22	15.55±2.22	31.11± 2.22	37.77±2.22
leave	10	8.88 ± 2.22	13.33±0.00	22.22± 2.22	38.88±2.22
	5	4.44 ± 2.22	8.88 ±2.22	11.11± 2.22	15.55±2.22
PBS		0.00 ± 0.00	0.00±0.00	0.00 ± 0.00	0.00 ±0.00
2 % DMSO		0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00	0.00 ±0.00
Albendazole (0.20 mg/ml)		100 ± 0.00	100 ±0.00	100 ± 0.00	100 ±0.00

Chemical analysis of the extracts from the *M.azedarach* revealed the presence of tannins, phenolic compounds, flavonoids, alkaloids, saponins and steroids (Dantas et al., 2000; Maciel et al., 2006; Sharma and Paul., 2013). Tannins are compounds noted for having anthelmintic properties. Tannins in the extracts could be the active component affecting the eggs and larvae of *H.contortus* (Athanasiadou et al., 2001) and they may operate through binding to free proteins, which lowers nutrient a vailability and leads to larval mortality by starvation, or attaching to the larval cuticle, abundant inglycoproteins, resulting in death. Alkaloid can influence the central nervous system, resulting in paralysis of the parasite and subsequently death (Roy et al.,2010), whereas saponin alters the permeability of the parasite's cell membrane, leading to vacuolization, tegument disintegration and finally death (Melzig et al., 2001). Flavonoid (isoflavones) blocks the glycolysis enzyme, disrupts calcium balance, hinders Nitrous Oxide function and leads to the parasite's evertual death (Stepek et al., 2006), while exhibiting low toxicity in mammalian animal hosts. Akhtar and Riffat (1984) assessed the effectiveness of *Melia azedarach* in com bating gastrointestinal nematodes in goats. They have indicated a 99.4=12 decrease in EPG in animals treated with *M.azedarach* fruit powder at a dosage of 30 mg/kg. Falbo et al. (2008) studied gastrointestinal nematodes in sheep, achieving an efficiency of 33.2%. Squires et al.(2010) indicated that in small ruminants, the rumen might act as a storage site, delaying the movement of the anthelmintic treatment and thereby extending *H. contortus'*s exposure to the active ingredient. The aqueous and hydroalcoholic extracts from the *Melia azedarach* leaves inhibited 99.4% and 100% of egg hatching, and fully stopped larval development at a concentration of 12.5 mg/ml respectively (Kamaraj et al., 2010).

Khanal et al. (2014) noted that *Swertia chirata* is also effective in fighting intestinal worms. The aqueous and hydroalcoholic extracts from the leaves blocked 99.4% and 100% of egg hatching, and completely prevented larval development at a concentration of 12.5 mg/ml respectively (Kamaraj et al., 2010). Iqbal et al., (2006) stated that in vitro investigation into the anthelmintic characteristics of Swertia chirata showed that at a concentration of 25 mg/ml, the crude aqueous extract from the entire *S.chirata* plant displayed an anthelmintic effect on live *Haemonchuscontortus.* PaezLeon et al., (2022) observed an 85.88% reduction in the hatching of *H. contortus*eggs at a concentration of 20 mg/ml after 48 hours of exposure. In a study, goats were given crude powdered and aqueous extracts of *Swertia chirata*at a dosage of 500 mg/kg body weight,orally for seven continuous days and results showed that *S.chirata* demonstrated considerable anthelmintic effectiveness against gastrointestinal nematodes namely *Bunostonairmspp.,Trichostrongyles spp., Oesophagostomum spp.,* and*Haemonchus spp.* account for about 70 to 90 percent (Jain and Sahni, 2009).

4. **CONCLUSION:**

It may be concluded that both Mahaneem (*Melia azedarach*) and Chirata (*Swertia chirata) have* the anthelmintic activity against H.contortus at different concentration. Chirata have better anthelmintic activity than Mahaneem. Both may be utilised after proper doseing against Haemonchuscontortus and other GINs in small ruminats as its easily avilable to poor farmers in different agroclimatic condition. However,these plant's anthelmintic activity on gastrointestinal nematodes in small ruminants remains to be clarified by in vivo experiments.

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